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14. ABSTRACT

Since the initiation of this proposal, we have demonstrated that while LPP1 and LPP3 mRNAlevels are decreased approximately 2 fold in breast cancer cells, autotaxin levels areincreased approximately 28 fold in breast cancer cells isolated directly from patients.This should result in increased LPA and S1P production by breast cancer cells in patients.Using a novel enzyme activity assay, we have demonstrated that autotaxin acitivity is notsignificantly different in blood from control and breast cancer patients. Thus, theincreased mRNA levels in tumor cells are not translated into increased autotaxin activityin the blood stream.We have demonstrated that downregulation of autotaxin by RNAi results in a decreasedsignaling, a novel S phase arrest and apoptosis in breast cancer cells.We have utilized a novel Si1P antibody to neutralize S1P in vitro and are currentlytreating mice with breast cancer xenografts to determine effects on cell growth.We have established transgenic mice expression the three LPA receptors as well asautotaxin in breast epithelium. We have obtained a LPP transgenic mouse to determine theeffects of degradation of LPA and S1P on breast function and tumorigenesis by crossing tothe above mice and to tumor prone mice.

15. Subject Terms (keywords previously assigned to proposal abstract or terms which apply to this award)
Autotaxin, lysophosphatidic acid, sphingosine 1 phosphate, lysoPLD

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Introduction
This application was originally for fellowship support of Makiko Umezu-Goto Dr. Makiko Goto, unexpectedly, due to family reasons and a job offer as faculty, returned to Japan in Nov 2003. The faculty position was in part due to her progress on this project and the award to the Fellowship grant demonstrating her excellence. Thus the goal of the award of training a Post Doctoral Fellow (PDF) and forwarding a career in breast cancer research was clearly furthered. Dr. Tanyi graciously took over these important studies. It is important to note that Dr. Tanyi was not supported financially from the grant. Again, due to the success of his studies related to this grant, Dr. Tanyi was offered a position in the residency program at Baylor College of Medicine in June 2004. Dr. Tanyi will complete his residency and then enter a translational research program. Once again, the goal of the award -- training a PDF and forwarding a career in breast cancer research -- was furthered. With this success, Dr. Shuying Liu took over this important project in Sept 2004 and we had the opportunity to develop a third career in breast cancer research.

Background: Multiple different bioactive lysophospholipids, including lysophosphatidic acid (LPA), lysophosphatidylcholine (LPC), sphingosylphosphorylcholine (SPC), sphingosine 1 phosphate (S1P) and lysophosphatidylserine (LPS) exhibit pleiomorphic effects on multiple cell lineages including breast cancer cells. LPA and S1P signal cells through specific cell surface receptors of the EDG family of cell surface G protein coupled receptors (GPCR), whereas SPC and LPC activate the OGR1 family of GPCR. Further LPC, LPS and LPA activate breast cancer cells as indicated by increases in tyrosine phosphorylation, cytosolic calcium, and phosphorylation of the p70S6, ERK and JNK kinases. The effects of LPA and LPC on intracellular signaling in breast cancer cells is translated in to functional changes such as increases in production of multiple growth factors from breast cancer cells including interleukin 6 and 8, which are potent regulators of neovascularization and activation of the AP-1 transcription complex. Goetzl and colleagues have demonstrated that lysophospholipids can increase the proliferation of breast cancer cells. In support of a role for lysophospholipids in signaling in breast cancer, multiple EDG receptors are aberrantly expressed in breast cancer cells.
The mechanisms regulating the production and degradation of lysophospholipids are just beginning to be elucidated. The most likely pathway for LPA production is the conversion of membrane phosphatidylcholine (PC) to LPC by the action of PLA1 or PLA2. LPC is converted to LPA by lysophospholipase D (lysoPLD) aka Autotaxin. Autotaxin is a major regulator of cellular motility and invasion. Further high levels of autotaxin correlate with aggressiveness and metastatic capacity of breast cancer cell lines. We have demonstrated that lysoPLD converts LPC to LPA and the resultant LPA induces cellular proliferation, cellular survival and cellular motility and chemotaxis. LPA, in turn, is degraded by lysophosphatidic acid phosphatases (LPP) to monoacylglycerol.

Objective/Hypothesis: An improved understanding of the production, metabolism and function of lysophosphatidic acid (LPA) and sphingosine 1 phosphate (S1P) in breast cancer could lead to the identification of novel markers or targets for therapy.

Specific Aims: (1) To determine the mechanisms regulating the production and metabolism of LPA and S1P in breast cancer.

(2) To determine the interplay between LPA and S1P in the proliferation, survival, invasion and metastases of breast cancer
(3) To determine whether the production or action of LPA and S1P are targets for therapy in breast cancer
Relevance: Lysophospholipids appear to play an important role in the initiation and progression of breast cancer. The enzymes producing these lysophospholipids as well as their receptors are targets for therapy in breast cancer.

Body
We have made significant progress on each of the aims in this proposal.
We have determined lysophospholipid phosphohydrolases (LPP1, 2, 3) and autotaxin (ATX) levels in breast cancer cell lines with QPCR and in breast cancer patients by transcriptional profiling (1). While LPP1 and LPP3 are decreased approximately 2 fold in tumor cells, ATX is increased approximately 28 fold in breast cancer cells directly from patients (1). This should result in increased LPA and S1P production by breast cancer cells in vivo.

Using a novel enzyme activity assay, we have demonstrated that autotaxin acitivity is not significantly different between sera and plasma from control and breast cancer patients (1). Thus, the increased mRNA levels in tumor cells are not translated into increased autotaxin activity in the blood stream (Makiko Goto performed these studies).

We have demonstrated that downregulation of autotaxin by RNAi results in a decreased signaling, a novel S phase arrest and apoptosis in breast cancer cells (Manuscript in preparation). We are currently determining the effects of over and underexpression of LPP1s in proliferation and survival of breast cancer cells in vitro. We will establish stable breast cancer cell lines with over or underexpression of ATX or LPPs and determine the effects on growth in vivo. If constitutive stable cell lines cannot be developed, we will develop conditionally expressing cell lines. We have developed a LPP expressing adenovirus and will determine the effects of “gene therapy” with this virus on in vivo tumor growth. (Studies in progress by Shuying Liu).

We have obtained a S1P neutralizing antibody. We have used this antibody to neutralize S1P in vitro and are currently treating mice with breast cancer xenografts to determine effects on cell growth (Studies in progress by Shuying Lu).

We have obtained a series of agonists and antagoinists of LPA receptors. With these agonists and antagonists, we have demonstrated that growth, motility and production of neovascularization factors including IL8, IL6 and VEGF is mediated by specific LPA receptors in breast cancer cells (Studies in progress by Shuying Liu).

We have established transgenic mice expression the three LPA receptors as well as autotaxin in breast epithelium. We have obtained a LPP transgenic mouse to determine the effects of degradation of LPA and S1P on breast function and tumorigenesis by crossing to the above mice and to tumor prone mice (The original constructs and mice were developed by Dr. Goto. The studies of the effects on tumorigenesis are in progress by Shuying Lu).

Key Research Accomplishments

1. LPP1 and LPP3 mRNA levels are decreased approximately 2 fold in breast cancer cells directly from the patient (Makiko Goto, Janos Tanyi)
2. Autotaxin mRNA is increased approximately 28 fold in breast cancer cells directly from patients (1). (Makiko Goto and Janos Tanyi)
3. Autotaxin acitivity is not significantly different between sera and plasma from control and breast cancer patients (1). (Makiko Goto)
4. Downregulation of autotaxin by RNAi results in a decreased signaling, a novel S phase arrest and apoptosis in breast cancer cells (Manuscript in preparation). (Makiko Goto)
5. We have demonstrated that neutralizing S1P in vitro decreases growth of some but not all breast cancer cell lines (Studies in progress by Shuying Lu).
6. We have established transgenic mice expression the three LPA receptors as well as autotaxin in breast epithelium. (The original constructs and mice were developed by Dr. Goto. The studies of the effects on tumorigenesis are in progress by Shuying Lu).
7. We have obtained a LPP transgenic mouse (Studies in progress by Shuying Lu).

Reportable outcomes

Umezu-Goto, M., Tanyi, J., Lahad, J., Liu, S., Yu, S., Lapushin, R., Hasegawa, Y., Lu, Y., Trost, R., Bevers, T., Jonasch, E., Aldape, K., Liu, J., James, R.A., Ferguson, C.G., Xu, Y., Prestwich, G.D., and Mills G.B., 2004 Lysophosphatidic acid production and action: Validated targets in cancer?. J. Cellular Biochemistry 92:1115-40.

The following manuscripts were facilitated by the support from this fellowship but are not directly reportable outcomes.
Tanyi JL, Morris AJ, Wolf JK, Fang X, Hasegawa Y, Lapushin R, Auersperg N, Sigal YJ, Newman RA, Felix EA, Atkinson EN, Mills GB.2003 The Human Lipid Phosphate Phosphatase-3 Decreases the Growth, Survival, and Tumorigenesis of Ovarian Cancer Cells: Validation of the Lysophosphatidic Acid Signaling Cascade as a Target for Therapy in Ovarian Cancer. Cancer Res. 63:1073-1082.
Tanyi, J.L., Morris, A.M., Wolf J.K., Bast R.C., Lu, K., Smith, D., Kalli K., Hartmann, L., McCune, K., Lu, K., Broaddus, R., Cheng, K.W., Atkinson, E.N., Yamal, J.M., Lapushin R., and Mills G.B., 2003 Role of decreased levels of LPP-1 in accumulation of lysophosphatidic acid (LPA) in ovarian cancer Clinical Cancer Res:. 9:3534-3545
Hasegawa, Y., Umezu-Goto, M. and Mills GB 2004 Lysophosphatidic acid (LPA) analogs, D-3-deoxy-phosphophatidyl-*myo*-inositol ether lipid (DPIEL) and lysophosphatidylglycerol (LPG), antagonize LPA receptor activation Submitted.

Conclusions The overarching goal of the training support was to train exciting new investigators in breast cancer research through validating S1P and LPA production and action as potential targets for therapy in breast cancer.

References

1. Umezu-Goto, M., Tanyi, J., Lahad, J., Liu, S., Yu, S., Lapushin, R., Hasegawa, Y., Lu, Y., Trost, R., Bevers, T., Jonasch, E., Aldape, K., Liu, J., James, R.A., Ferguson, C.G., Xu, Y.,

Prestwich, G.D., and Mills G.B., 2004 Lysophosphatidic acid production and action: Validated targets in cancer?. J. Cellular Biochemistry 92:1115-40.

Appendix - not attached but available in the open literature

Umezu-Goto, M., Tanyi, J., Lahad, J., Liu, S., Yu, S., Lapushin, R., Hasegawa, Y., Lu, Y., Trost, R., Bevers, T., Jonasch, E., Aldape, K., Liu, J., James, R.A., Ferguson, C.G., Xu, Y., Prestwich, G.D., and Mills G.B., 2004 Lysophosphatidic acid production and action: Validated targets in cancer?. J. Cellular Biochemistry 92:1115-40.